

### **REMARKS**

Applicant respectfully requests reconsideration.

Applicant has renumbered claims 16-98 (previously numbered 17-99) to correct a typographical error in the previous claim numbering. The claim numbering referred to in this amendment is based on the corrected numbers.

Claims 1-20, 22, 27-32, 43, 45-57, 63-65, 70-73, 76-80, 83, 84, 88, 89, 94, 95, 97 and 98 were previously pending in this application. Claims 14-15, 45-57, 63-65, 70-73, 76-80, 83-84, 88-89, 94-95 and 97-98 have been withdrawn.

Claims 16-98 have been amended to correct claim numbering and claim dependencies.

As a result, claims 1-13, 16-20, 22, 27-32, and 43 are pending for examination with claim 1 being an independent claim.

No new matter has been added.

### **Objections**

The Examiner objected to the numbering of the claims. Applicant has renumbered claims 16-98 (previously numbered 17-99) to correct a typographical error in the previous claim numbering.

Reconsideration and withdrawal of this objection is respectfully requested.

### **Rejection under 35 U.S.C. §112, First Paragraph**

The Examiner rejected claims 1-13, 16-20, 27-32 and 43 under 35 U.S.C. §112, first paragraph as failing to comply with the enablement requirement. Applicant respectfully traverses.

Applicant wishes to stress that the pending claims are composition claims. MPEP 2164.01(c) states that for a composition claim that is not limited by a recited use (i.e., a use recited in the claim), “any enabled use that would reasonably correlate with the entire scope of that claim is sufficient to preclude a rejection for nonenablement based on how to use”. Furthermore, “if any use is enabled when multiple uses are disclosed, the application is enabling for the claimed invention”.

Id. The specification discloses multiple uses for the claimed compositions. For example, the specification clearly states that the claimed nucleic acids stimulate immune responses in vitro and in

vivo, and the Examples section demonstrate that the claimed nucleic acids can be used to generate antibodies in mice. These recited and demonstrated uses are sufficient to demonstrate enablement of the rejected composition claims, under the standard set forth in MPEP 2164.01(c). The claimed compositions are therefore enabled.

The enablement requirement is satisfied if one of ordinary skill in the art is able to make and use the claimed invention without undue experimentation, based on the specification and the knowledge in the art at the time of filing. The experimentation required to make and use the claimed invention may be complex, and still not undue, if the art routinely engages in that level of experimentation. Multiple factors may be considered in analyzing whether an invention is enabled including 1) the nature of the invention; 2) the breadth of the claims; 3) the state of the art; 4) the level of ordinary skill in the art; 5) the level of predictability in the art; 6) the amount of direction provided by the inventor(s); 7) the existence of working examples; and 8) the quantity of experimentation needed to make or use the invention based on the content of the disclosure. In re Wands, 858 F.2d 731; 8 USPQ 2d 1400 (Fed. Cir. 1988). These factors are to be considered in their totality with no one factor being dispositive. The analysis of these factors as presented below illustrates that the experimentation required to practice the invention is not undue.

Nature of the invention: The invention relates to immunostimulatory nucleic acids that minimally comprise the defined core 24 nucleotide sequence of SEQ ID NO:1. These nucleic acids are, by definition, immunostimulatory. They are able to stimulate an innate immune response as well as an adaptive immune response.

Breadth of the claims: The rejected claims relate to compositions of nucleic acids comprising the defined core nucleotide sequence (i.e., SEQ ID NO:1).

Level of ordinary skill in the art: One of ordinary skill in the art would be familiar with nucleic acid synthesis, formulation and administration to human or non-human subjects.

State of the Art and Predictability in the Art: The Examiner asserts that while the art recognizes the importance of Th1 type immune responses, no particular Th1 associated cytokine has been determined to be responsible for ameliorating viral infection. The Examiner contends that “cytokines can be inherently toxic, have unclear pharmacological behavior and also have pleiotropic effects” making their use unpredictable.

The pending claims relate to compositions comprising immunostimulatory nucleic acids. As disclosed in the specification, CpG-containing nucleic acids have a multi-faceted effect on the immune system, including “inducing B cell proliferation; cytokine and immunoglobulin secretion; natural killer (NK) cell lytic activity and IFN- $\gamma$  secretion; and activation of dendritic cells (DCs) and other antigen presenting cells to express costimulatory molecules and secrete cytokines, especially the Th1-like cytokines that are important in promoting the development of Th1-like T cell responses.” See page 1 lines 25-29. The specification discloses IFN- $\gamma$ , IL-12, TNF- $\alpha$  and IFN- $\alpha$  as examples of Th1 type cytokines. The immune stimulation induced by an immunostimulatory nucleic acid is distinct from that induced by administering a single cytokine in isolation, as is the case in several of the references cited by the Examiner. A CpG-containing immunostimulatory nucleic acid induces an immune response similar to that induced by a pathogen, inducing physiological levels of multiple cytokines. This is distinguishable from administering high levels of one cytokine in isolation, which would not result in physiological cytokine levels. Furthermore, immunostimulatory nucleic acids do not only affect the Th1 type immune response, but in fact affect the balance between Th1 and Th2 immune responses. As disclosed on page 27 lines 28-30, “immunostimulatory nucleic acids...shift the immune response in a subject from a Th2 ...to a Th1 response.” Thus, the specification discloses that immunostimulatory nucleic acids have a multi-faceted effect on the immune system and induce physiological levels of multiple different cytokines.

The Examiner cites Infante-Duarte et al. (*Springer Semin Immunopathol* (1999) 21:317-338) for the teaching that “in addition to a Th1 type immune response, a Th2 type immune response is also necessary” to control intracellular infection. The Examiner focuses on one example provided by Infante-Duarte et al. of an infection that requires a Th2 response (gastrointestinal helminths). However, when this reference is examined as a whole, the disclosure in fact emphasizes the importance of a Th1 response to intracellular pathogens. For example page 318 discloses that infection by the intracellular parasite *Leishmania major* is “the prototypic example of Th1-dependent protection and Th2-mediated susceptibility.” On page 319, leprosy is disclosed as another example in which the “development of a Th1 response is necessary for cure, and the development of a Th2 response is harmful.” Page 324 states “The typical response to intracellular pathogens is Th1.” Infante-Duarte et al. does not suggest that the claimed nucleic acid compositions

would not be immunostimulatory. In fact, on page 328 the authors state, in the context of protective effects of infections early in life, “Similar effects could be obtained by coadministration of allergen and CpG oligonucleotides.” Thus, overall this reference indicates that the art was aware of the importance of Th1 immune responses in responding to intracellular infection.

The Examiner further asserts that “the efficacy of Th1 associated cytokines...against intracellular pathogens are controversial”, and cites several references in support of this. Aoki et al. (*Expert Opin Emerg Drugs* (2004) 9:223-236) is cited for the teaching that interleukin 2 protects against a non-pathogenic mycobacterial strain but not against a virulent infection. However, Aoki et al. in fact emphasizes the importance of Th1 immunity. Page 224 discloses that Th1 cells “contribute to the elimination of intracellular pathogens such as mycobacterium and virus.” Page 225 states “Th1 immunity is able to eliminate leprosy bacillus.” Page 227 states that Th1 immune activation “is desired for host defence against many intracellular infectious diseases.” Page 228 states that “Type I immunity is essential to host defence” against *M. tuberculosis*. Page 231 states that “the Th1 environment also contributes to optimal protection against fungal infection.” Thus Aoki et al. does not call into question the importance of Th1 immunity, nor the immunostimulatory capacity of the claimed nucleic acid compositions. The Examiner has focused on the disclosure in Aoki et al. related to administration of one particular cytokine, interleukin 2. However the pending claims relate to compositions comprising immunostimulatory nucleic acids. The specification discloses that administration of immunostimulatory nucleic acids results in secretion of multiple cytokines, as well as exerting additional stimulatory effects on the immune system. None of the pending claims is limited to administration of interleukin 2 alone. Thus, nothing in Aoki et al. suggests that the claimed nucleic acid compositions would not be immunostimulatory.

The Examiner cites Bohn et al. (*Infect Immune* (1998) 66:2213-2220) for the teaching that interleukin-12 “induces different effector mechanisms that result in either protection or exacerbation of a disease.” Bohn et al. investigated the mechanisms exerted by IL-12 in mice that had specific genotypes, and were infected with *Yersinia*. The Examiner focuses on the disclosure in Bohn et al. of instances where interleukin-12 was observed to exacerbate *Yersinia* infections. However, this effect was only observed in a specific scenario in mice that were resistant to *Yersinia* infection. In non-resistant mice, IL-12 was found to protect against *Yersinia* infection. Regardless, as discussed

above, the pending claims relate to compositions comprising immunostimulatory nucleic acids. The specification discloses that administration of immunostimulatory nucleic acids results in secretion of multiple cytokines, as well as exerting additional stimulatory effects on the immune system. None of the pending claims is limited to administration of interleukin-12 alone. Thus, nothing in Bohn et al. suggests that the claimed nucleic acid compositions would not be immunostimulatory.

The Examiner cites Sakao et al. (*Int Immunol* (1999) 11:471-480) for the teaching that interleukin 18 is “responsible for the progression of endotoxin-induced liver injury in mice primed with interleukin 18.” Sakao et al. specifically relates to the role of interleukin 18 in (LPS)-induced liver injury, which is not relevant to the pending claims. None of the pending claims is limited to administration of IL-18 alone. Rather, the pending claims relate to compositions comprising immunostimulatory nucleic acids. Nothing in Sakao et al. suggests that the claimed nucleic acid compositions would not be immunostimulatory.

The Examiner cites Zaitseva et al. (*Blood* (2000) 96:3109-3117) for the teaching that “interleukin 6 and interferon gamma augment the susceptibility of monocyte-derived macrophages to infection.” However, Zaitseva et al. goes on to say that “IFN- $\gamma$  inhibited viral entry and productive infection of MDMs with macrophage-tropic (M-tropic) HIV-1.” (*See Abstract*). Furthermore, the experiments presented in Zaitseva et al. involve exposing cells to IL-6 or IFN- $\gamma$  individually and in the absence of other factors. None of the pending claims is limited to administration of IL-6 or IFN- $\gamma$  alone. Rather, the pending claims relate to compositions comprising immunostimulatory nucleic acids. The specification discloses that administration of immunostimulatory nucleic acids results in secretion of multiple cytokines, as well as exerting additional stimulatory effects on the immune system. Nothing in Zaitseva et al. suggests that the claimed nucleic acid compositions would not be immunostimulatory.

The Examiner cites Masihi (*Expert Opin Biol Ther* (2001) 1:641-653) for the teaching that “interleukin 2 increases the production of HIV in vitro, and enhances the translocation of bacteria from intestines to other organs in animal studies.” Masihi discusses a range of immunomodulatory agents. The Examiner focuses on one disclosure in Masihi related to the use of IL-2 in AIDS patients. However, as discussed above, none of the pending claims are limited to administration of a single cytokine alone. The overall teachings of Masihi in fact strongly support the pending

claims. Pages 646-647 disclose the use of CpG oligodeoxynucleotides in immune activation, stating “CpG dinucleotides...play an important role in innate immunity.” The authors further state “it is possible to use CpG DNA as an immunomodulator for therapeutic applications ...”. The reference provides multiple examples where administration of CpG oligodeoxynucleotides provided protection against pathogens. Moreover, the authors state on pages 646-647 “CpG oligodeoxynucleotides alone gave partial protection against leishmania infection and enhanced survival in combination with soluble leishmania antigen.” These teachings are in keeping with the pending claims which relate to compositions comprising immunostimulatory nucleic acids.

Applicant reiterates that the claims are composition claims and as such, “any enabled use that would reasonably correlate with the entire scope of that claim is sufficient to preclude a rejection for nonenablement based on how to use”. See MPEP 2164.01(c). Applicant has shown that the claimed oligonucleotides can be used to stimulate an immune response including the production of antibodies in mice. The Examiner hasn’t provided any rationale for why the claims are not enabled in view of such a showing.

The Examiner cites Krieg et al. (*Annu Rev Immunol* (2002) 20:709-760) and Mutwiri et al. (*Veterinary Immunol Immunopathol* (2003) 91:89-103) for the teaching that the “Th1 associated cytokine profile for ... oligonucleotides vary from one oligonucleotide and species of subject to the next.” The Examiner refers to a disclosure on page 716-717 of Krieg et al. which discusses qualitative differences between CpG oligonucleotides in terms of their capacity to induce TNF- $\alpha$ , and which discloses that different CpG molecules need to be considered separately. However, the pending claims do not relate to *any* oligonucleotides but rather to a group of related oligonucleotides that share the common sequence motif of SEQ ID NO:1, which has been demonstrated to be highly immunostimulatory. Furthermore, as disclosed in the specification, immunostimulatory CpG nucleic acids lead to the secretion of multiple cytokines, not just TNF- $\alpha$ . This specific disclosure referred to by the Examiner in Krieg et al. does not indicate that fluctuation in induction of TNF- $\alpha$  necessarily correlates with a fluctuation in immune stimulation in general. Thus nothing in Krieg et al. suggests that the claimed nucleic acid compositions would not be immunostimulatory.

Mutwiri et al. relates to the use of immunostimulatory CpG oligonucleotides in domestic animals. The Examiner alleges that Table 1 of Mutwiri et al. indicates that “in vitro

immunostimulatory activity of oligonucleotides containing the CpG motif varies from one species to the next.” However according to the accompanying legend for Table 1, a “plus” sign in the table indicates that a CpG ODN effect was detected, while a “minus” sign indicates that no effect was detected. A blank space indicates that the parameter was not tested in a given species. There are no “minus” signs on the table, except for in the context of a “±” sign. The Examiner appears to be incorrectly interpreting empty spaces on the table to be negative results. The Examiner also refers to a disclosure on page 93 of Mutwiri et al. related to expression of TLR9. While Mutwiri et al. discloses that there are some differences in expression of TLR9 between mice and humans, CpG ODN are known to be immunostimulatory in both mice and humans. Furthermore, while Mutwiri et al. discloses that TLR9 has not yet been identified in other species besides mice and humans, the authors go on to state “it is assumed that a similar signalling mechanism is involved in other species.” Thus nothing in Mutwiri et al. suggests that the claimed nucleic acid compositions would not be immunostimulatory in humans and in other species.

The collection of references cited by the Examiner demonstrate that the art was well aware of the beneficial immunostimulatory properties of CpG nucleic acids and of the importance of Th1 type immune responses to pathogens.

Amount of direction provided by the inventor(s): The Examiner asserts that there is a “lack of guidance in the specification concerning the effective use of the claimed invention.” Applicant respectfully disagrees.

Applicant has taught how to make and use the invention. The nucleotide sequence of SEQ ID NO:1 is provided. The art was familiar with how to make nucleic acids of a defined or random sequence and of a particular backbone composition. The combination of a nucleic acid and another agent such as an antigen, an adjuvant, an anti-microbial agent, and the like, would also be clear based at least on the teachings in the specification which provides examples of each category of agent to be combined with the claimed nucleic acids. A person of ordinary skill in the art would know how to make such combinations.

Applicant teaches how to use the invention. The specification teaches how to formulate, dose and administer the claimed nucleic acids, and to whom to administer the claimed nucleic acids.

The ability of a number of CpG immunostimulatory nucleic acids to stimulate immune responses was known at the time of filing.

Working examples: The claimed invention relates to immunostimulatory compositions comprising the defined core nucleotide sequence (i.e., SEQ ID NO:1). Immunostimulatory properties of such nucleic acids are demonstrated by the data presented in the Example section. The Examples show, *inter alia*, the ability of the claimed nucleic acids to activate B cells *in vitro* as indicated by proliferation (FIGs. 3 and 10) and surface marker activation (FIG. 2), to stimulate secretion of cytokines such as IFN-alpha, IP-10 and IL-10 by PBMC *in vitro* (FIGs. 4-9 and 11), to induce NK lytic activity *in vitro* (FIG. 12), and to stimulate antigen-specific antibody production *in vivo* when administered with an antigen (FIGs. 13-14). Thus the Examples demonstrate that the claimed nucleic acids can stimulate immune responses *in vivo* and *in vitro*, in the presence and absence of antigen, in human and mouse cells.

The Examiner asserts that the “specification does not set forth any examples with regard to using the claimed composition further comprising an microbial/viral antigen, anti-microbial agent, or anti-viral agent” and nor does the specification “predict or teach any positive therapeutic benefit of the composition.” Applicant respectfully disagrees.

As described on page 91 lines 11-23, page 94 lines 1-4 and FIGs. 13-14, SEQ ID NO:1 (CpG 10105) “significantly enhanced antibody titers against HBsAg compared to antigen alone” (see page 94) when coadministered with HBsAg (hepatitis B antigen). Thus, administering an oligonucleotide comprising SEQ ID NO:1 with a viral antigen significantly increases production of antibodies specific for that antigen.

Moreover, the specification discloses multiple uses for the claimed compositions. For example, the specification states that the claimed nucleic acids can be used to stimulate immune responses *in vivo* and *in vitro*, such as stimulating B-cells, NK T cells, and antigen-specific antibodies. *See*, FIGs. 3, 10, 12, 13 and 14. The specification further *demonstrates* immune stimulation by the claimed compositions, including *in vitro* and *in vivo* antigen specific and antigen non-specific responses. *See* Examples and FIGs. 1-14.

*Quantity of experimentation needed to practice the invention:* The Examiner asserts that due to lack of guidance in the specification one would not be able to practice the invention without undue experimentation.

As discussed above, Applicant has taught how to make and use the invention. One of ordinary skill in the art would be aware of how to obtain nucleic acids comprising SEQ ID NO:1. Furthermore, one of ordinary skill would be aware of how to use such nucleic acids for immune stimulation based on the disclosure in the specification and the level of general knowledge in the art. In view of the teaching of the instant application and the state of the art at the time of filing, the quantity of experimentation required to practice the method of the rejected claims is no greater than that in which the art routinely engages.

In view of the foregoing, the rejected claims can be practiced without undue experimentation and therefore the claims are enabled. Reconsideration and withdrawal of the rejection is respectfully requested.

**CONCLUSION**

A Notice of Allowance is respectfully requested. The Examiner is requested to call the undersigned at the telephone number listed below if this communication does not place the case in condition for allowance.

If this response is not considered timely filed and if a request for an extension of time is otherwise absent, Applicant hereby requests any necessary extension of time. If there is a fee occasioned by this response, including an extension fee, that is not covered by an enclosed payment, please charge any deficiency to Deposit Account No. 23/2825.

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Respectfully submitted,

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